

## Mouse Anti-HDM IgG2a Antibody Assay Kit

Catalog # 3038

For Research Use Only - Not Human or Therapeutic Use

### INTRODUCTION

Asthma is a common chronic inflammatory disease that affects 300 million people of all ages worldwide (1). It is caused by exposure to allergens such as dust mites, pet dander, pollen, or mold, and characterized by airflow obstruction and bronchospasm. House dust mite (HDM) is the most common asthma allergen, which affects up to 85% of asthma patients (2, 3). Of the two main mite species, *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f), more than 20 types of HDM allergens are defined based on sequential and functional homologies. Among those HDM allergens, group 1 (Der 1) and group 2 (Der 2) dominate overall allergic responses in patients and are the most commonly researched allergens (4-6).

Previously, asthma was considered to be an inflammatory airway disease mediated by the adaptive immune system, particularly type 2 helper T-cells (7). However, recent studies indicate that the innate immune system is also involved in triggering an inflammatory response in both asthma patients and animal models (8-10). These inflammatory changes and airway remodeling significantly vary depending on the types of allergens (11). To meet such needs, a mouse HDM-induced asthma model is a useful tool to dissect the pathological roles of the adaptive and innate immune systems activated by different HDM elements. This is an advantage over the classic ovalbumin-induced asthma model which activates adaptive immunity preferentially.

Recently, it was reported that HDM-specific sublingual immunotherapy (SLIT) is more efficacious than subcutaneous immunotherapy at preventing the development of allergic inflammatory reactions in a mouse model (12). This SLIT protocol has been approved as a treatment to reduce allergy or asthma symptoms in patients (13).

To study the immune response to allergens and allergen-specific pathological effects in mouse models, Chondrex, Inc. provides mouse ELISA kits for assaying anti-HDM IgG, IgM, and IgE antibodies (Catalog # 3030, 3036, 3037), in addition to anti-HDM antibody IgG subclasses IgG1, IgG2a, IgG2b, and IgG3 (Catalog # 3034, 3038, 3035, 3039). Chondrex, Inc. also offers ELISA kits for assaying anti-OVA antibody subtypes IgA, IgE, IgG, and IgM and anti-OVA IgG subclasses IgG1, IgG2a, IgG2b, and IgG2c, (Catalog # 3004, 3010, 3011, 3015 - 3018, 3029) as well as total immunoglobulin subtypes IgA, IgE, IgG, and IgM, and IgG subclasses IgG1, IgG2a, IgG2b, and IgG3, (Catalog # 3005, 3019, 3023 - 3028).

Note: Other antibody subtype ELISA kits against HDM as well as HDM antigen detection kits are currently under development. Please contact Chondrex, Inc. support (support@chondrex.com) for more information.

### KIT COMPONENTS

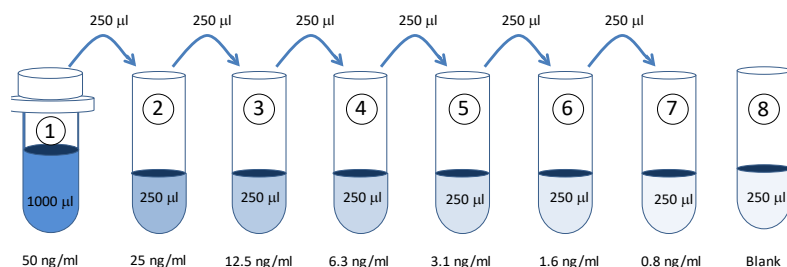
| Item                                                                              | Quantity | Amount                                | Storage |
|-----------------------------------------------------------------------------------|----------|---------------------------------------|---------|
| Standard Anti-HDM Mouse IgG2a Antibody (30381)                                    | 1 vial   | 50 ng/vial, lyophilized               | -20°C   |
| Secondary Antibody - Peroxidase-Conjugated Goat Anti-Mouse IgG2a Antibody (30383) | 2 vials  | 50 µl                                 | -20°C   |
| Solution B - Blocking Buffer (30313)                                              | 1 bottle | 10 ml                                 | -20°C   |
| Solution C - Sample/Standard/Detection Antibody Dilution Buffer (30314)           | 1 bottle | 50 ml                                 | -20°C   |
| TMB Solution (90023)                                                              | 2 vials  | 0.2 ml                                | -20°C   |
| Chromogen Dilution Buffer (90022)                                                 | 1 bottle | 20 ml                                 | -20°C   |
| Stop Solution - 2N Sulfuric Acid (9016)                                           | 1 bottle | 10 ml                                 | -20°C   |
| Wash Buffer, 20X (9005)                                                           | 1 bottle | 50 ml                                 | -20°C   |
| HDM from <i>Dermatophagoides pteronyssinus</i> Coated ELISA Plate                 | 1 each   | 96-well<br>(Brown 8-well strips x 12) | -20°C   |

## NOTES BEFORE USING ASSAY

- Note 1: It is recommended that the standard and samples be run in duplicate.
- Note 2: Warm up all buffers to room temperature before use.
- Note 3: Partially used reagents may be kept at  $-20^{\circ}\text{C}$ .
- Note 4: Crystals may form in the 20X wash buffer when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are dissolved completely.
- Note 5: Measure exact volume of buffers using a serological pipette, as extra buffer is provided.
- Note 6: Cover the plate with plastic wrap or a plate sealer after each step to avoid the edge effect.

## ASSAY PROCEDURE

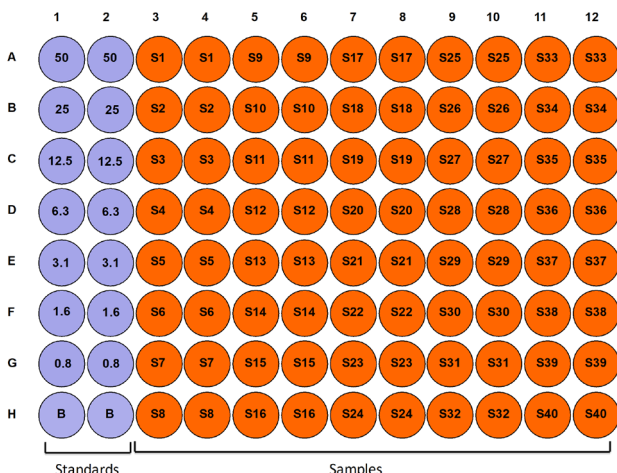
- Dilute Wash Buffer:** Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer).
- Add Blocking Buffer:** Add 100  $\mu\text{l}$  of the Blocking Buffer (Solution B) to each well and incubate for 1 hour at room temperature.
- Prepare Standard Dilutions:** The recommended standard range is 0.8 - 50 ng/ml. Dissolve one vial of Standard (50 ng/vial) in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution C) and keep it as a 50 ng/ml standard stock. Add 250  $\mu\text{l}$  of this standard solution to 250  $\mu\text{l}$  of Solution C to make a 25 ng/ml solution. Then, serially dilute it with Solution C. For example, mix 250  $\mu\text{l}$  of the 25 ng/ml solution with an equal volume of Solution C to make a 12.5 ng/ml solution, and then repeat it five more times for 6.3, 3.1, 1.6, and 0.8 ng/ml standard solutions.



- Prepare Sample Dilutions:** The dilution of serum from mouse immunized with HDM varies (1:10 or more) depending on the immunization schedule and timing of serum collection. In general, no IgG2a antibodies against HDM are observed in normal serum at a 1:10 dilution.
- Wash:** Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

6. **Add Standards and Samples:** Add 100  $\mu$ l of standards, Solution C (blank), and samples to wells in duplicate. Incubate at room temperature for 2 hours.

Figure 1 - Mouse anti-HDM IgG2a Assay Standard layout.



7. **Wash:** Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*
8. **Add Secondary Antibody:** Dissolve one vial of Secondary Antibody in 10 ml Sample/Standard/Detection Antibody Dilution Buffer (Solution C). Add 100  $\mu$ l of detection antibody solution to each well and incubate at room temperature for 1 hour.
9. **Wash:** Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*
10. **Add TMB:** Use new tubes when preparing TMB. Dilute one vial of TMB with 10 ml Chromogen Dilution Buffer just prior to use. Add 100  $\mu$ l of TMB solution to all wells immediately after washing the plate and incubate for 25 minutes at room temperature.
11. **Stop:** Add 50  $\mu$ l of 2N sulfuric acid (Stop Solution) to each well.
12. **Read Plate:** Read the OD values at 450 nm (a 630 nm filter can be used as a reference). If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution.

## CALCULATION OF ANTIBODY TITERS

1. Average the duplicate OD values for the standards, blanks (B), and test samples.
2. Subtract the averaged blank (B) values from the averaged OD values of the standards and test samples.
3. Plot the OD values of the standards against the ng/ml of antibody standard. Using a log/log plot will linearize the data. Figure 2 shows an example of a standard curve of anti-HDM IgG2a antibodies.
4. The ng/ml of antibody in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the antibody concentration (ng/ml) in original sample specimens.

Figure 2 - A typical standard curve for anti-HDM IgG2a assay

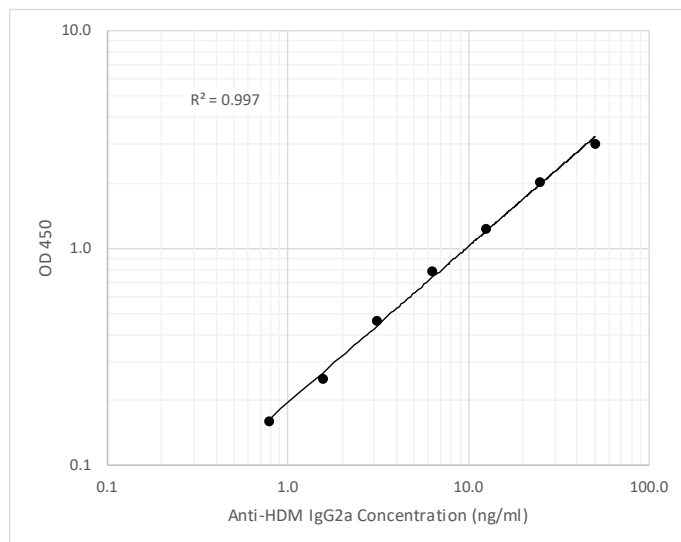


Table 1 - Reproducibility of data assayed by Mouse Anti-HDM IgG2a Antibody Assay Kit

| Test At            | 1.6 ng/ml | 6.3 ng/ml | 25 ng/ml |
|--------------------|-----------|-----------|----------|
| Inter-Assay CV (%) | 7.4       | 10.8      | 11.5     |
| Intra-Assay CV (%) | 6.3       | 4.4       | 2.7      |
| Spiking Test*      | 87%       | 92%       | 96%      |

Known amounts of anti-HDM IgG2a were added to samples and then diluted with Sample/Standard/Detection Antibody Dilution Buffer to assay anti-HDM IgG2a antibodies by ELISA.

## REFERENCES

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